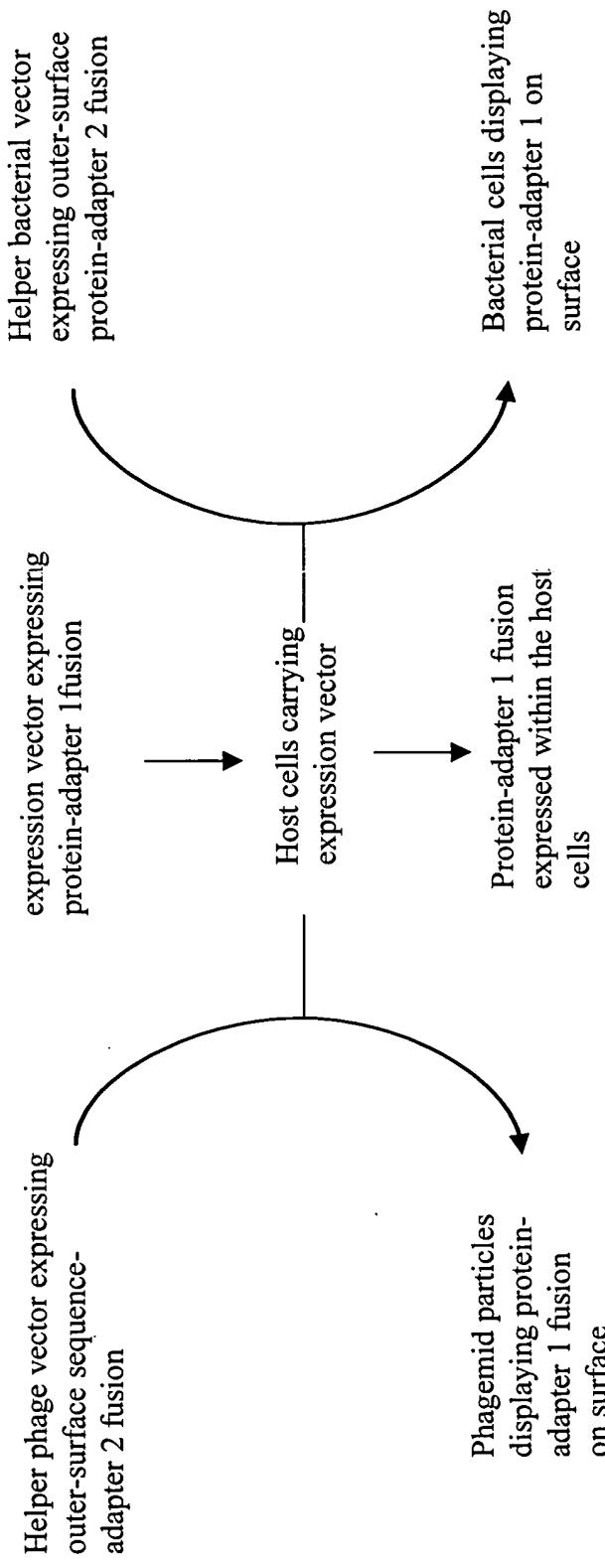


Adapter-directed display systems

TO 20 T T " 65 E E E D D T



Phage display

Protein expression

Bacterial display

Fig. 1

KO7kpn phage Screening by ELISA

TO 2014 "SIEGE E COOL

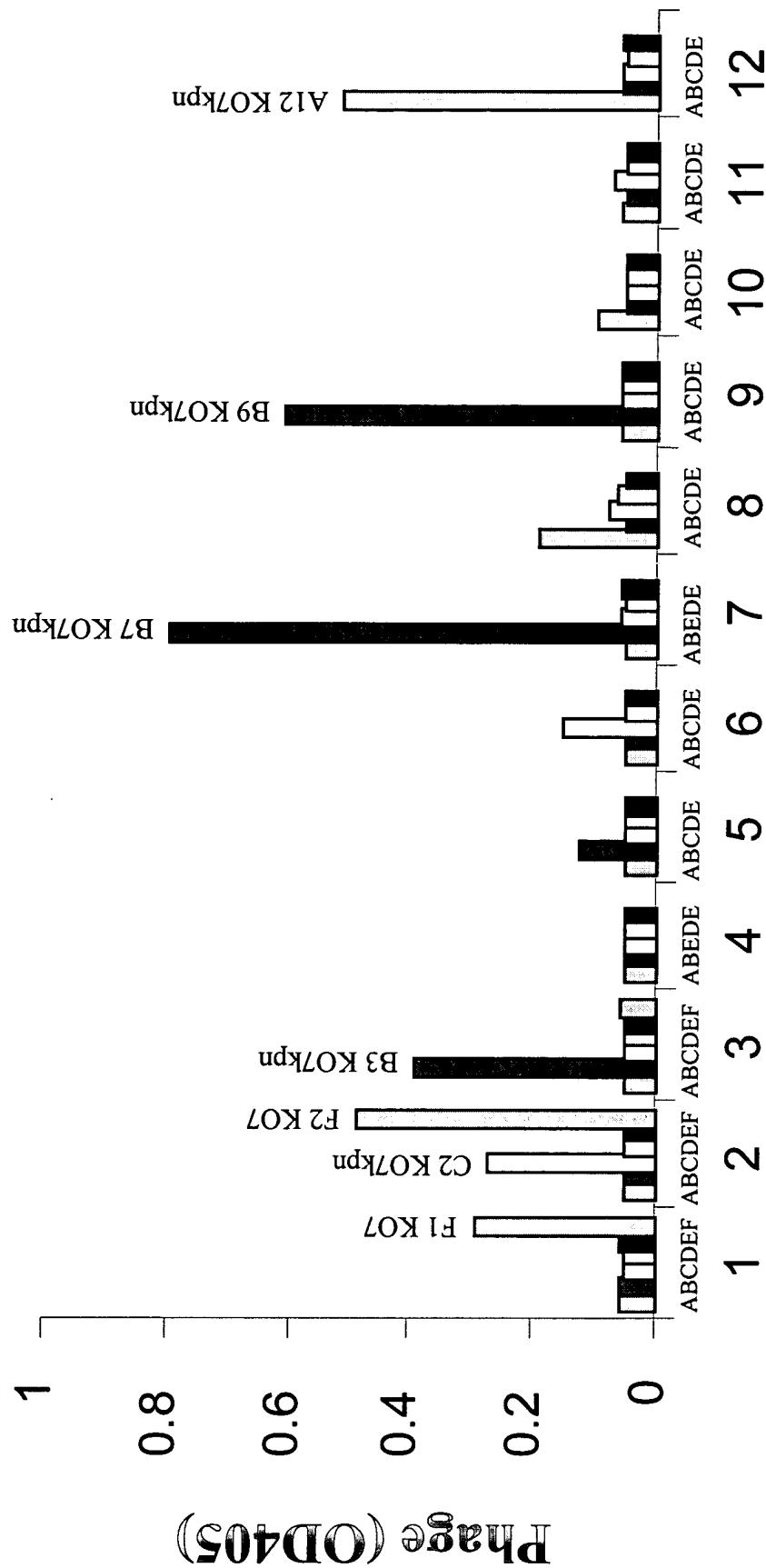


Fig. 2

KO7kpn helper phage Vector

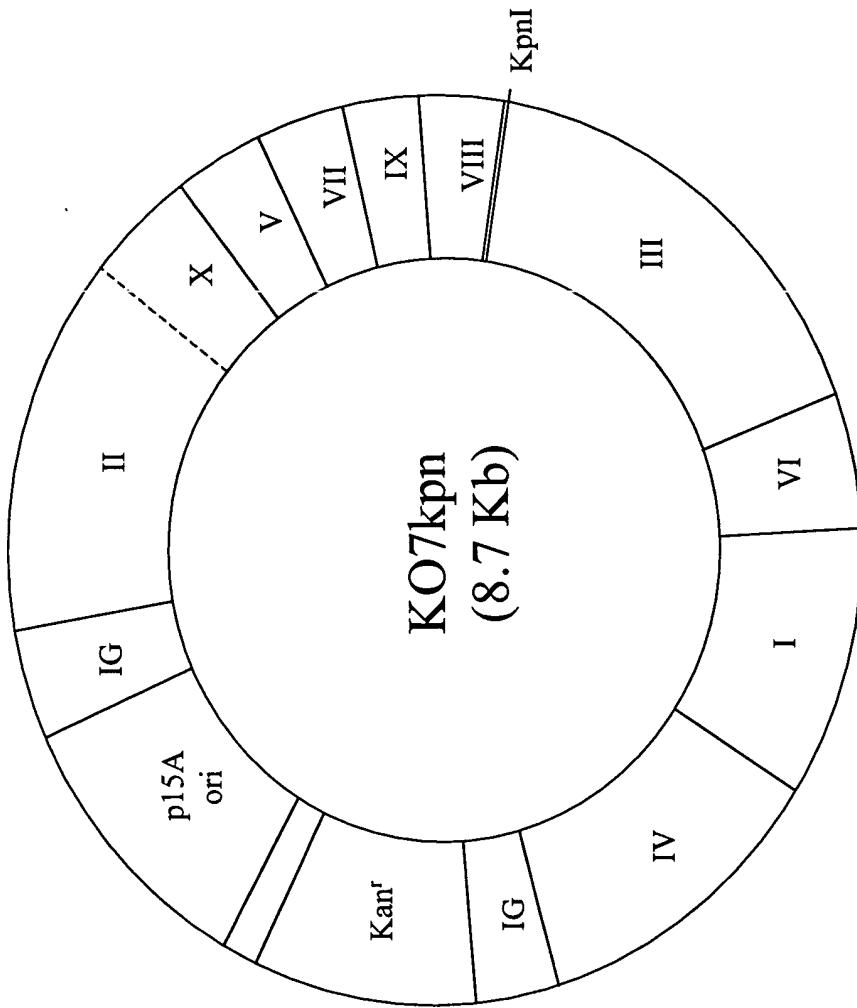


Fig. 3A

Gene III leader sequence in KO7 helper phage

GTG AAA TAA TTA TTC GCA ATT CCT TTA GTT CCT TTC TAT TCT CAC TCC GCT
V K L L F A I P L V V P F Y S H S A

Gene III leader sequence in KO7kpn helper phage

KpnI
GTG AAA AAA TAA TTA TTC GCA ATT CCT TTA GTA CCT TTC TAT TCT CAC TCC GCT
V K L L F A I P L V V P F Y S H S A

Map of phagemid vector pABMC6

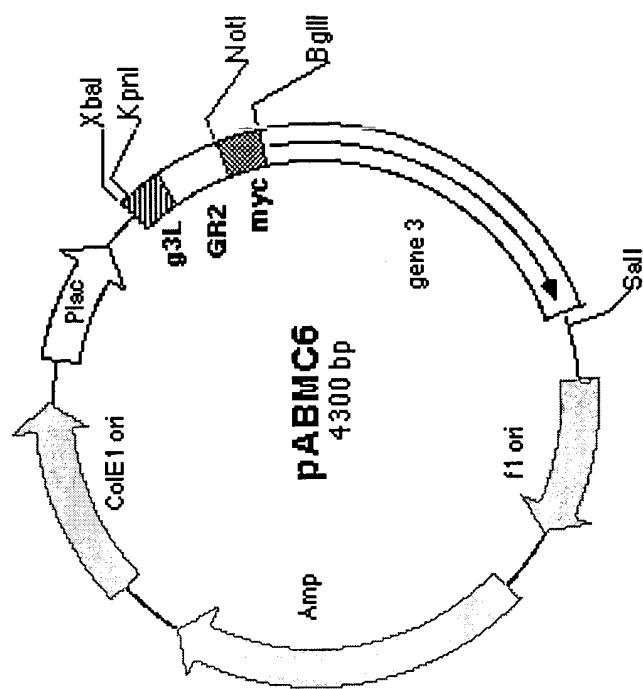


Fig. 4

Helper phage with engineered gene III fused to adaptor 2

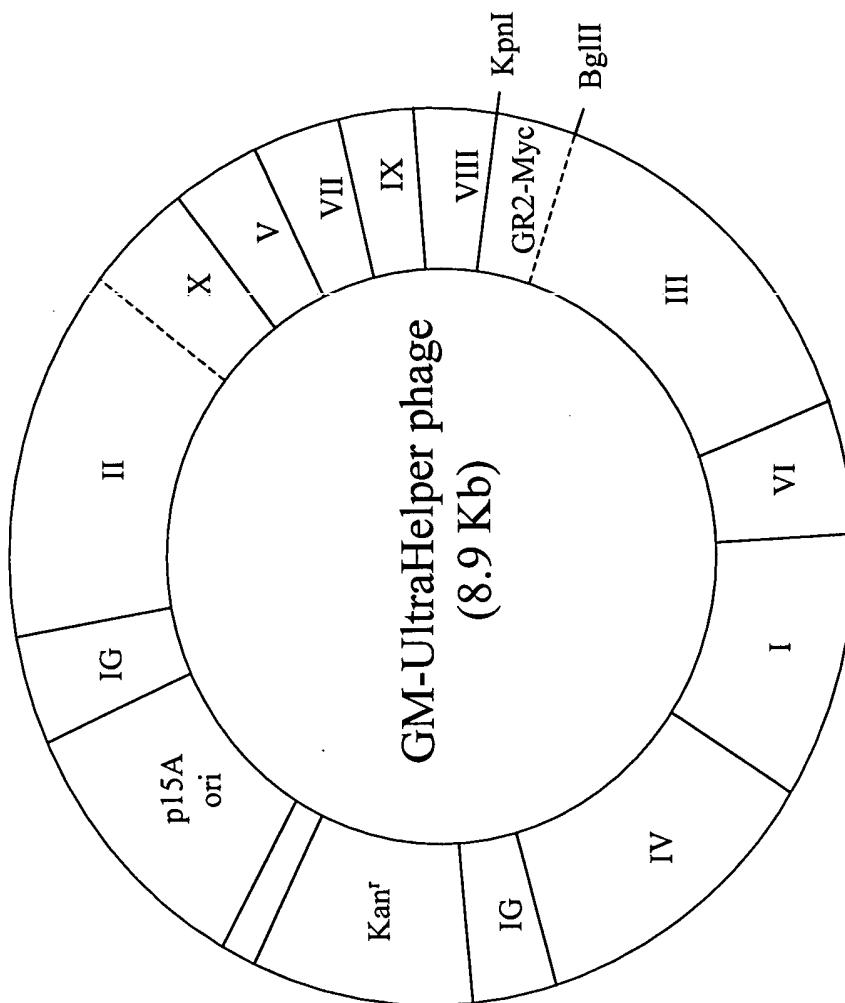


Fig. 5A

GR2-Myc domain coding sequence in GM-UltraHelper phage genome

KpnI Gene III leader GR2
-TTAGTGGTACCTTCTATTCCTCACTCCGCT ACATCCCCCTGGAGGGCTTACAGTCAGAAACCATGCCCTGCGA
- L V V P F Y S H S A T S R L E G L Q S E N H R L R

NotI
ATGAAGATCACAGAGCTGGATAAGACTTGGAAAGAGGTCAACCATGCAGGTGCAGGGACGGTGGAGGTTGC GGGCCGCA
M K I T E L D K D L E E V T M Q L Q D V G G C A A A A

Myc-tag BglII Gene III
GAACAAAAACTCATCTCAGAAGAGGATCTG AGATCTGGAGGGGT ACTGTTGAAAGTTGTTAGCAAA---
E Q K L I S E E D L R S G G T V E S C L A K -

Fig. 5B

T S R L E G L Q S E N H R L R M K I T E L D K D L E E V

GR2 domain

T M Q L Q D V G G C A A A E Q K L I S E E D L R S G G G

Myc-tag

Trypsin cleavage sites at GR2-Myc domain on GM-UltraHelper phage

Fig. 5C

GR2-Myc-pIII fusions assemble into GM phage particles

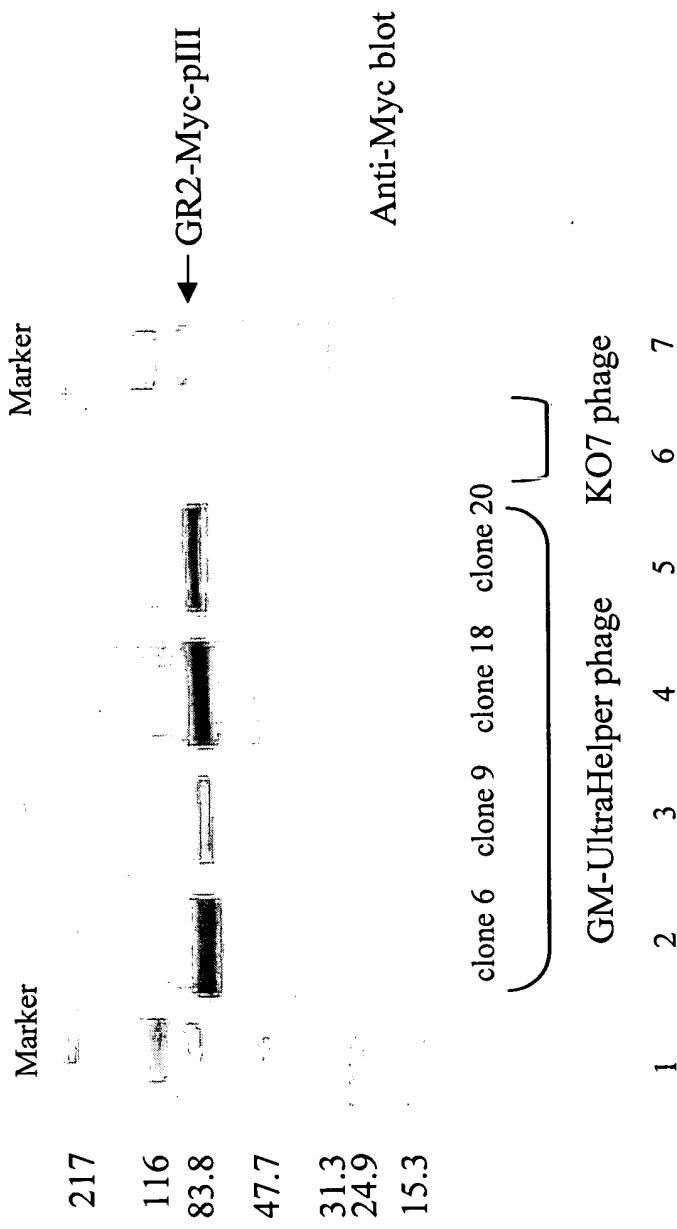


Fig. 6

Detection of GR2-Myc domain on GM-UltraHelper phage

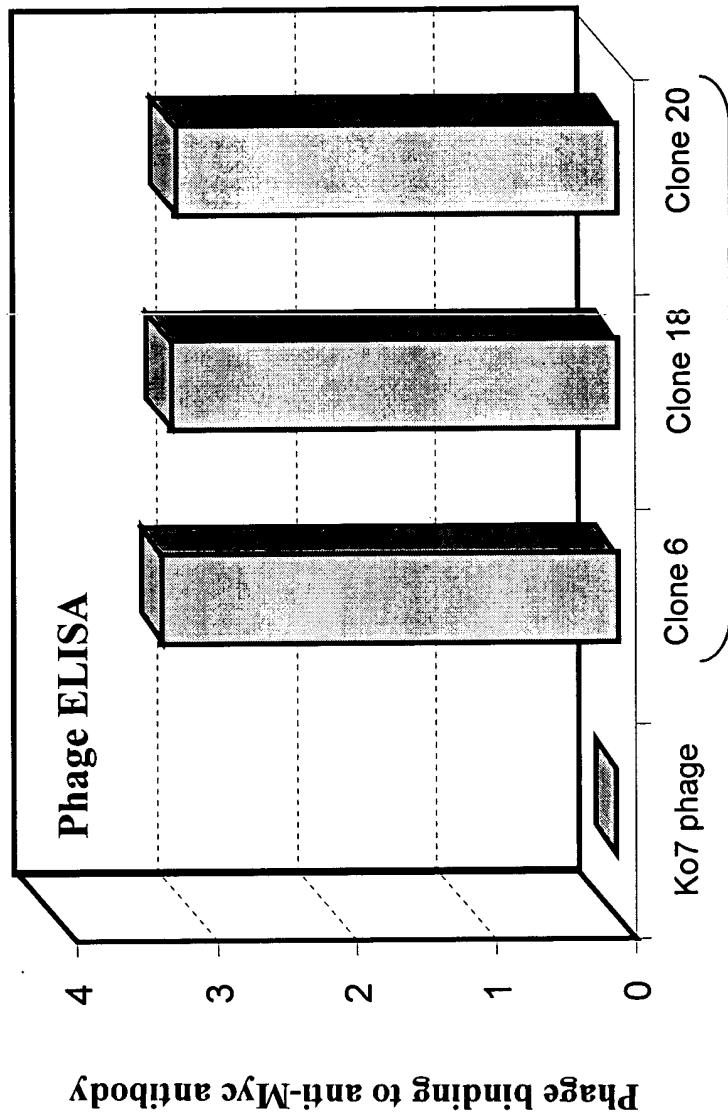


Fig. 7

TO 20 TT" \$5EEEE00T

Cleavage of GR2-Myc domains on GM phages by trypsin

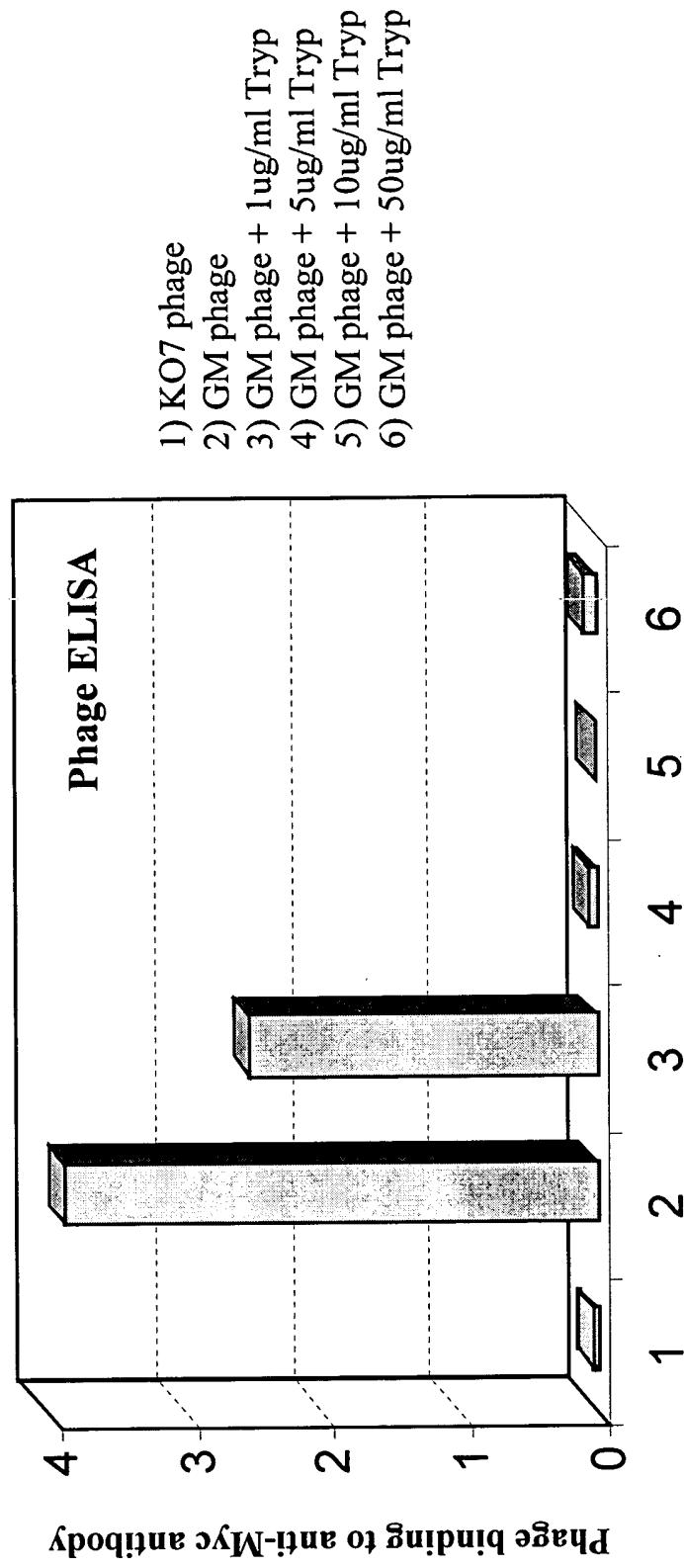


Fig. 8

Phagemid vector for protein-GR1 expression

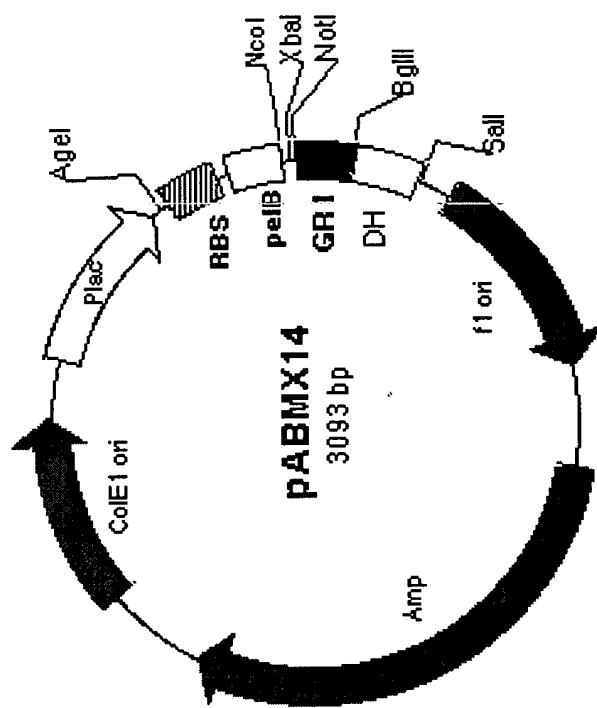


Fig. 9A

Complete vector sequence of pABMX14

Functional display of scFv by GM-UltraHelper phage

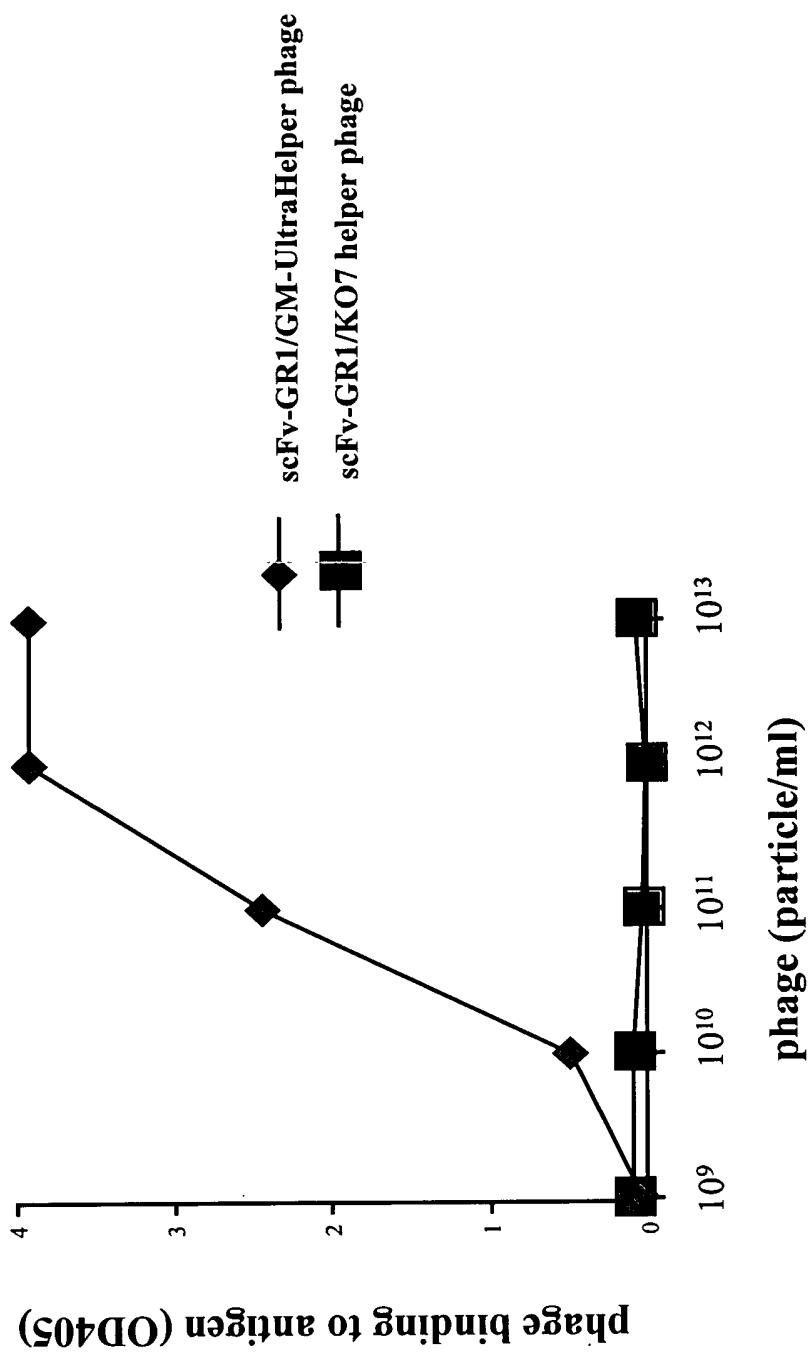
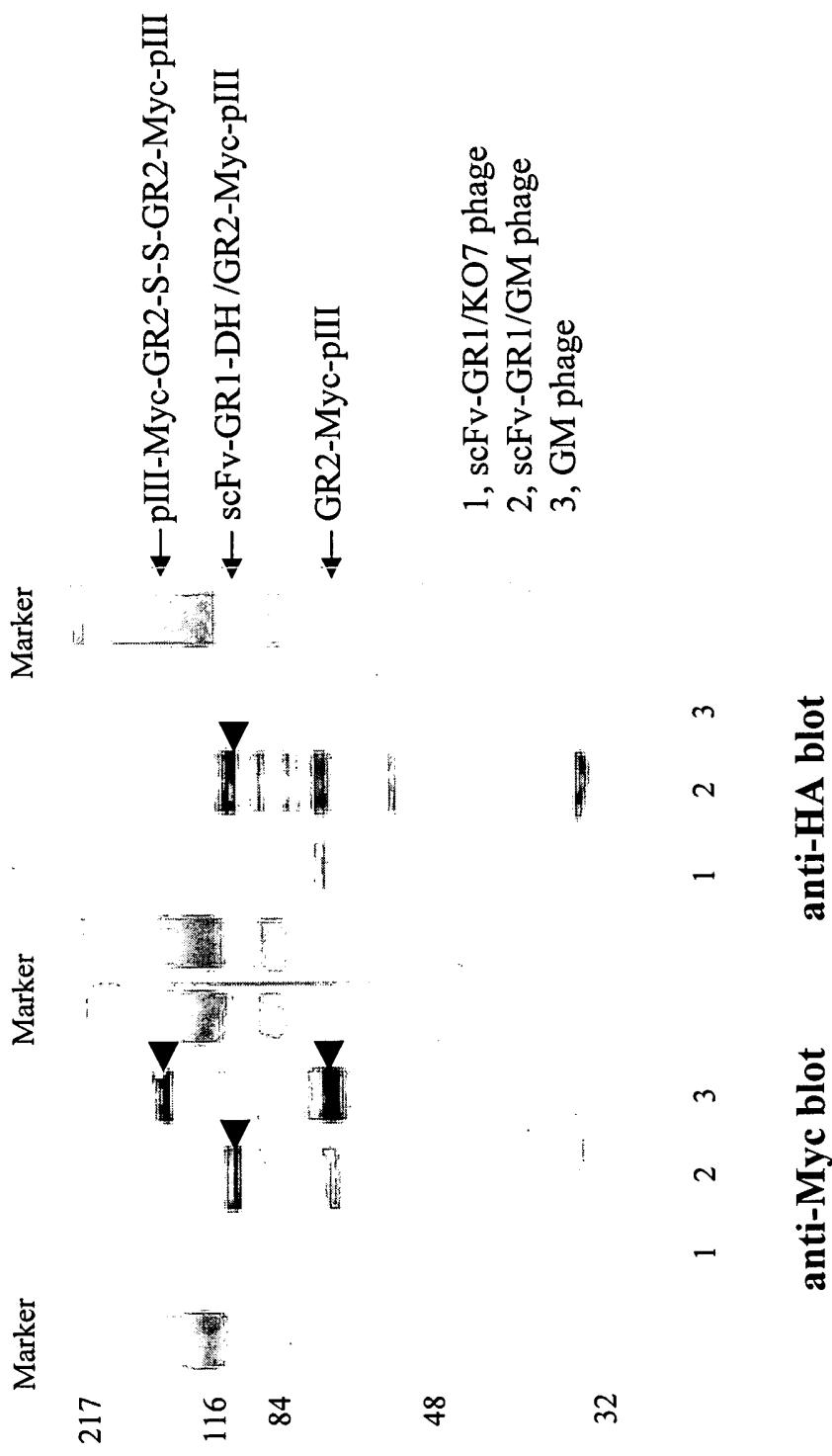


Fig. 10

Mutivalent display of scFv by GM-UltraHelper phage



Map of phagemid vector pABMC13

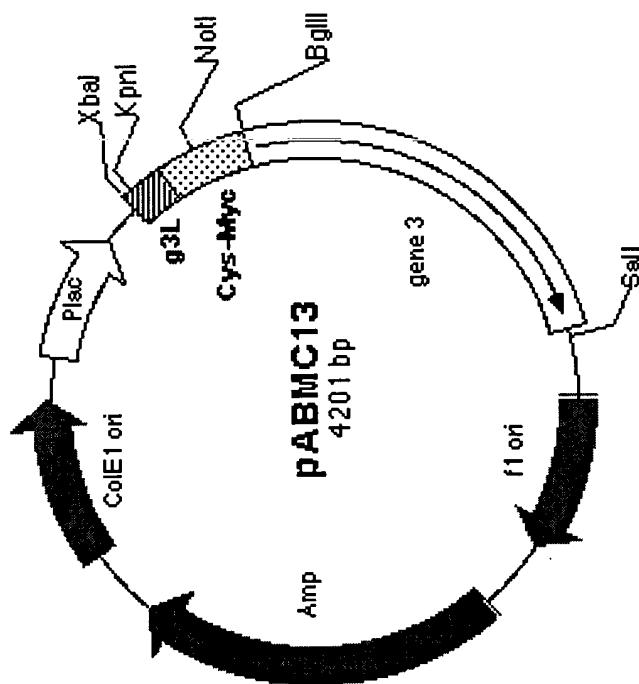


Fig. 12

Helper phage with Cys-Myc-pIII fusion gene

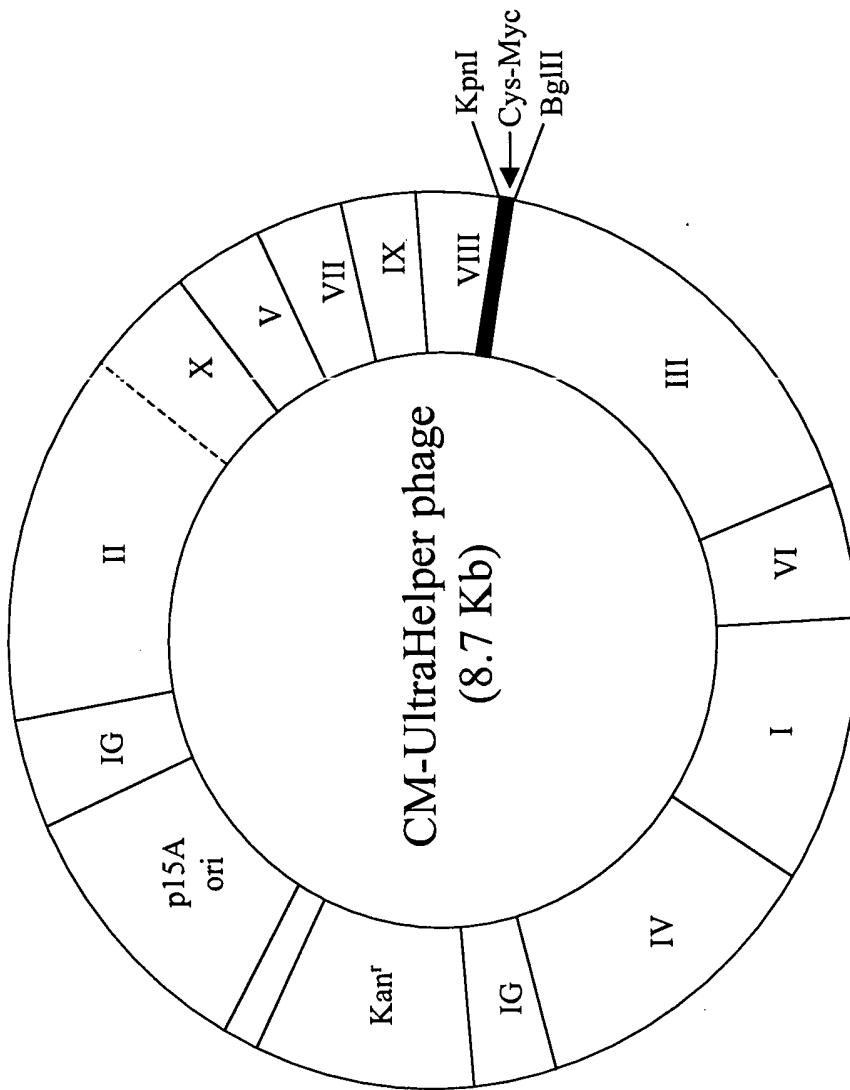


Fig. 13A

T C C C T T " Gene III

Engineered gene III sequence in CM phage

KpnI Gene III leader Amber stop NotI Myc-tag BgIII
- - - T T A G T G G T A C C T T C T A T T C T C A T C T C C G C T T A G G C T G C G G G T G G G C A G A A C A A A A A C T C A T C T C A G A A G G G A T C T G A G A T C T A G A T C T G G A
- L V V P F Y S A * A C G G A A E Q K L I S E D L R S R S G

Gene III
G G C G G T A C T G T G A A A G T T G T T A G G C A A A A C C T C A T A C A G A A A A T T C A T T A C G T C T G G A A A C A C G G A C A A A A C T T A G A T C G T T A C G C T - - -
G G T V E S C L A K P H T E N S F T N V W K D D K T L D R Y A - -

Fig. 13B

Detection of Myc-tag on CM-UltraHelper phages by ELISA

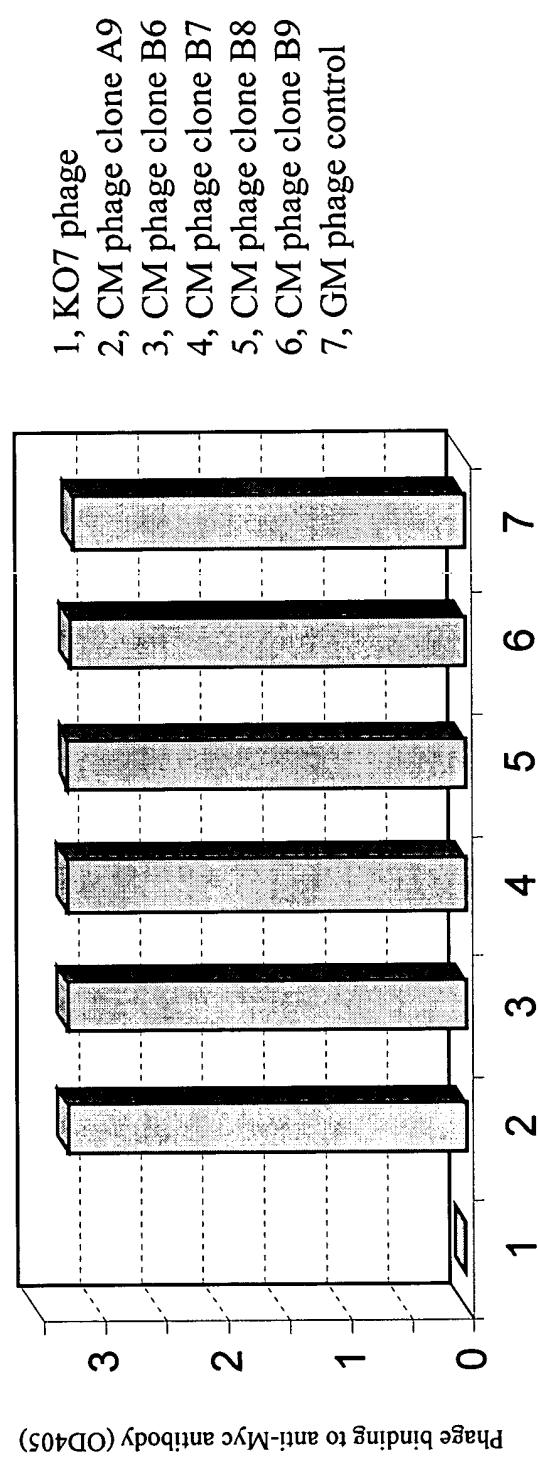


Fig. 14

Phagemid vector for protein-HA-cys expression

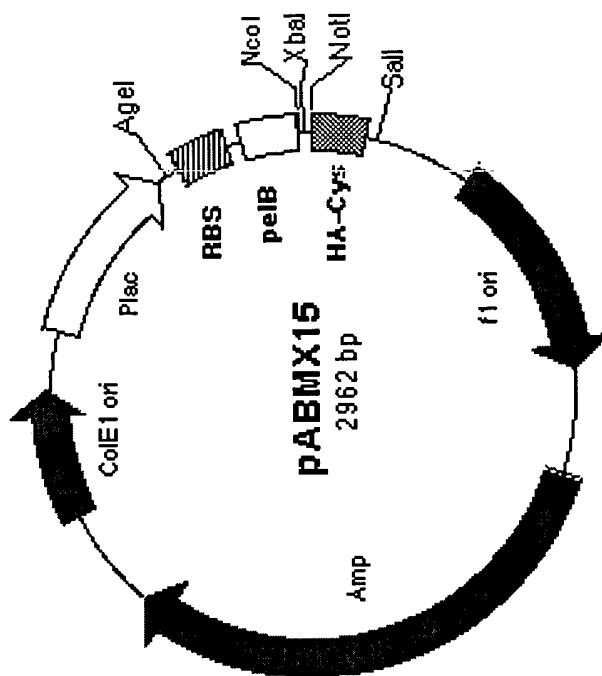


Fig. 15A

Complete vector sequence of pABMX15

GCGCAACGCCAAATTAAATGTGAGTTAGCTCACTCATAGGCCACCCAGGCTTACACTTATGCTTCCGGCTCGTATGTTGAGGGATAACAAATTACCGGTCTTTAACTTTAGTAAGGGAGA
 ATTAAAATGAAATACCCATTGCTTACGGCTGCGTGGATTGTTATTACTCGGGCCAGCCGGCCATGGGGCCTCTAGGGCGCTTACCGGTACGACCTTCGGACTCGCAGGGTGGCT
 GCTGATAAGTCGACCTCGACCAATTGCCCCATAGTGAGTCGTTATTACATTACTCGGGCGTGTGAGTCGTTAAGTCACGTCGTTACCCAACTTAATCGCCCTTGAGCA
 GCGAGCTGGCTGAATAGCGAGGGCCCTCCCAAGCTGCGAGCTGAGGGCTTCCCACTGGAGAATGGGAAGGGCCCTGTAGACTGGGGTTACGGGTCTCCCTAGGGTCTCAGGCT
 GCTACACTTGGCCAGGGCCATGGCTTCCCTCTCTGGCTTCCCACTGGCACTGGCTTCCCACTGGCTTCCCACTGGCTTCCCACTGGCTTCCCACTGGCTTCCCACTGGCT
 CTCGACGCCAAAAACTGATTAGGGTGGCTTACGGCTGGCTTGGCTTGGCTTACGGCTGGCTTGGCTTACGGCTGGCTTGGCTTACGGCTGGCTTACGGCTGGCTTACGGCT
 AACCCCTATCTCGGCTTATTCTTGTGATTATAAGGGATTGCTGATTGGCTTGGCTTGGCTTGGCTTGGCTTGGCTTGGCTTGGCTTGGCTTGGCTTGGCTTGGCTTGGCT
 CTTTTCGGGAAATGTGCGCCGAAACCCCTATTGGTTATTCTAAATACATTCAAAATATGTATCCCTCATGAGACATAACCCCTATAAATGCTCAAAATAATGAAAAGGAGTAA
 ATTCGGTGTGGCCCTTATTCCCTTGTGGCTTGGCTTGGCTTGGCTTGGCTTGGCTTGGCTTGGCTTGGCTTGGCTTGGCTTGGCTTGGCTTGGCTTGGCTTGGCTTGGCT
 ACAGGGTAAGATCCTGAGGTTTGGCCCGAAGAACCTTCCAAATGAGGACTTTAAAGTCTGAGCTTAAAGTCTGAGCTTAAAGTCTGAGCTTAAAGTCTGAGCTTAAAGTCTGAGCT
 ATTCTGAGATGACTGG
 TGGGGGACGGAAAGGGCTAACCGCTTGGCAACATGGGGATCATGTAACCTGGCTGATGTAACCTGGCTGAAATGAGCCATACCCAAACGACGCGGTGACACCAAGTGG
 CAACAAACGTTGGCACAACATTAACCTGAGCTTACTCTGAGCTTACTCTGAGCTTACTCTGAGCTTACTCTGAGCTTACTCTGAGCTTACTCTGAGCTTACTCTGAGCT
 CTGATAAAATCTGGAGCCTGG
 TGCTGAGATGGTGTCTCACTGAGCTGTAAGCTGAGCTGTAAGCTGAGCTGTAAGCTGAGCTGTAAGCTGAGCTGTAAGCTGAGCTGTAAGCTGAGCTGTAAGCTGAG
 TGACCAAAATCCCTTAACGCTGAGCTGG
 CGGTGGTTGGTGGCTGGGATCAAGGTAACTCTGTTACGGCTGAGCTGGCTGAGCTGGCTGAGCTGGCTGAGCTGGCTGAGCTGGCTGAGCTGGCTGAGCTGGCT
 CGCCTACATACCTCGCT
 GCACACAGCCAGCTGGAGCTCCAGGGAAACGCTGAGCTGGCTGGGTTGGCTGAGCTGGCTGAGCTGGCTGAGCTGGCTGAGCTGGCTGAGCTGGCTGAGCTGGCT
 AGGCACAGGGAGCTGG
 CGGCTTTAGGTTCTGGCTTGTGGCTTGTGGCTTGTGGCTTGTGGCTTGTGGCTTGTGGCTTGTGGCTTGTGGCTTGTGGCTTGTGGCTTGTGGCTTGTGGCT
 AGGCAGGGAGCTGGCT

Functional display of scFv by CM-UltraHelper phage

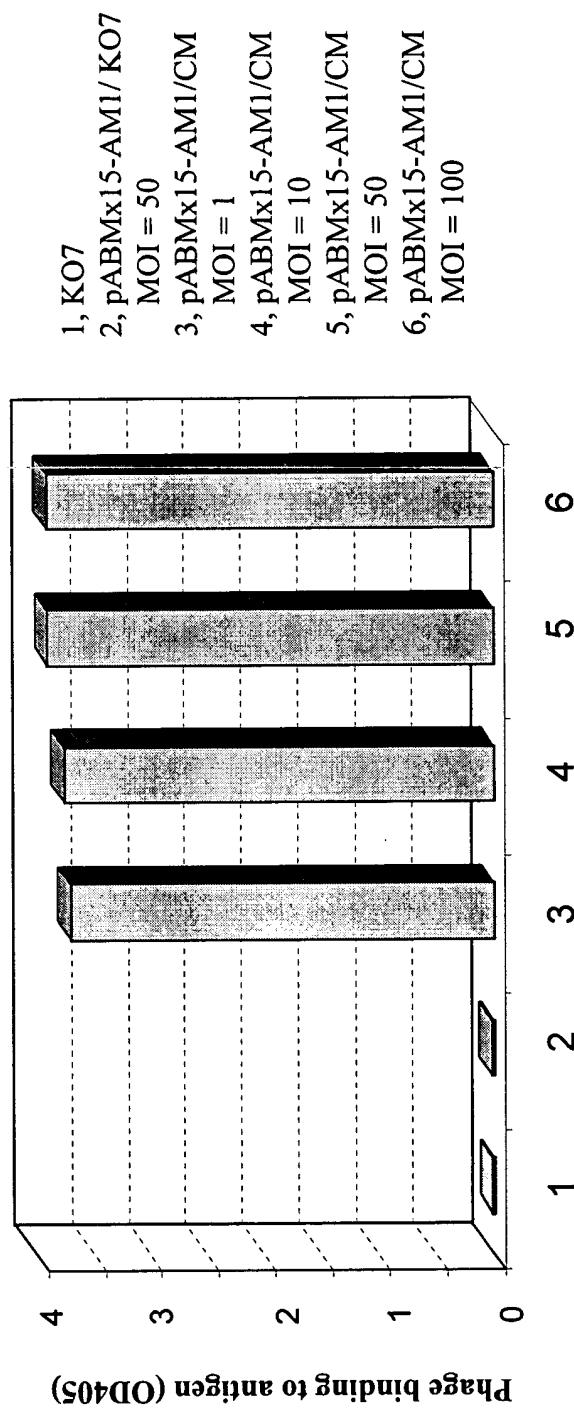
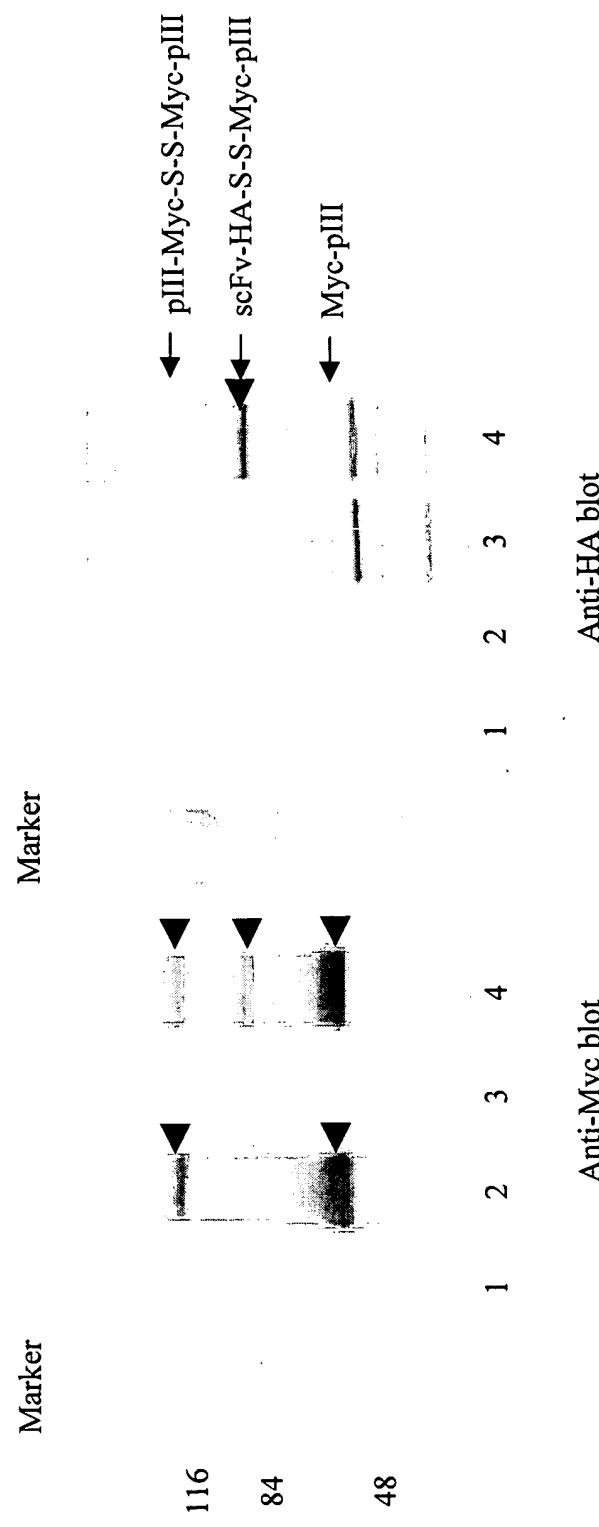


Fig. 16

Detection of scFv displayed by CM-UltraHelper phage



1: KO7 phage; 2: CM phage; 3: pABMx15-AM1/ KO7; 4: pABMx15-AM1/CM

Fig. 17

Map of phagemid vector pABMC12

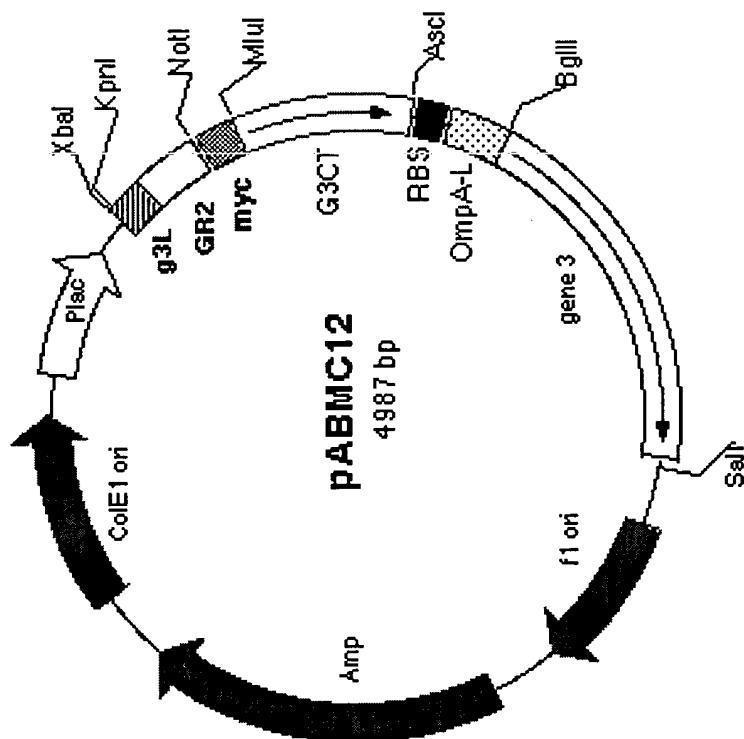


Fig. 18

Helper phage with an additional copy of engineered gene III

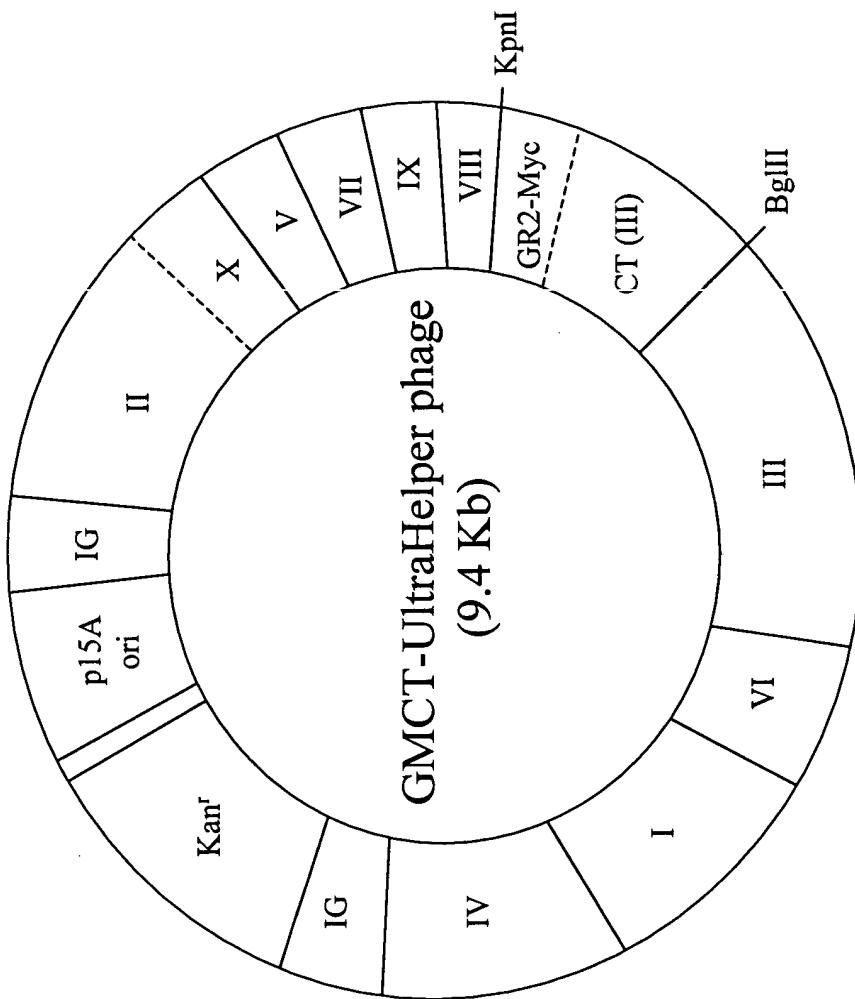


Fig. 19A

Engineered gene III Sequence in GMCT phage genome

Fig. 19B

Functional display of scFv by GMCT-UltraHelper phage

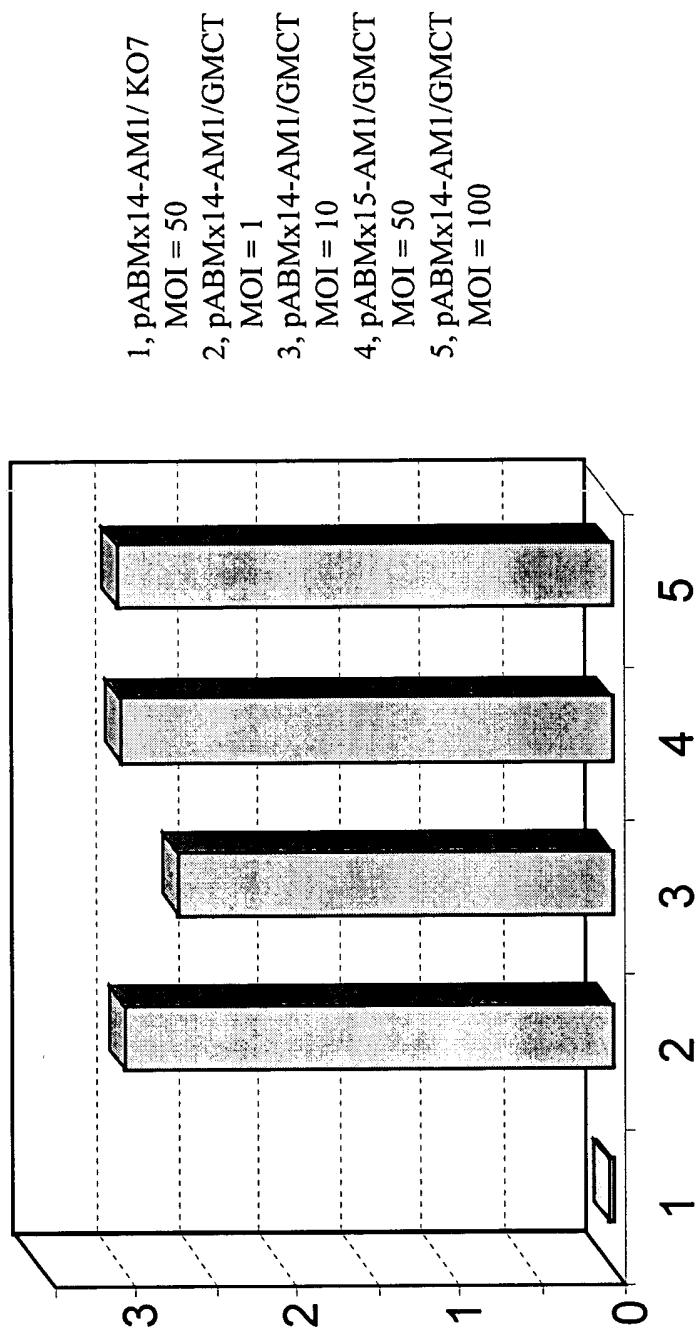


Fig. 20

Detection of scFv displayed by GMCT-UltraHelper phage

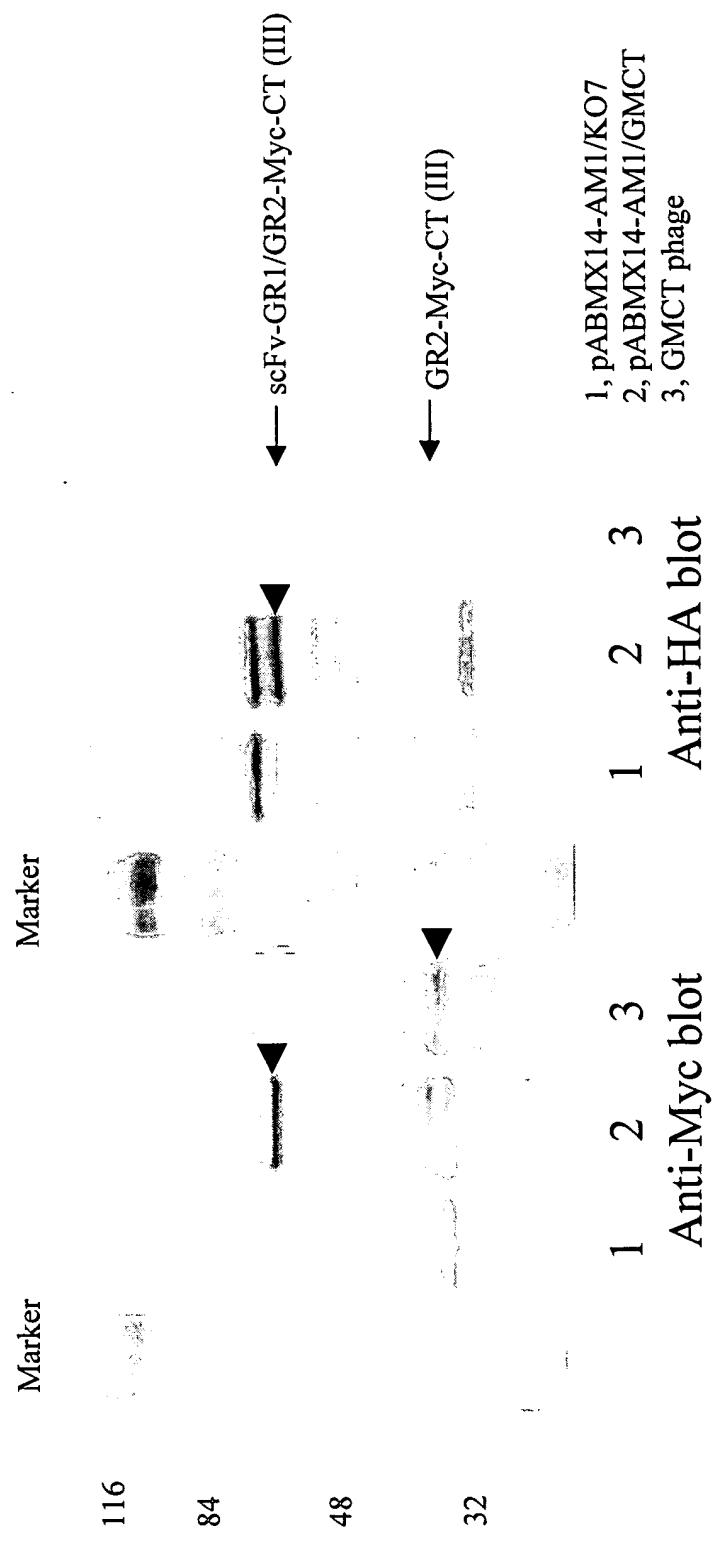


Fig. 21

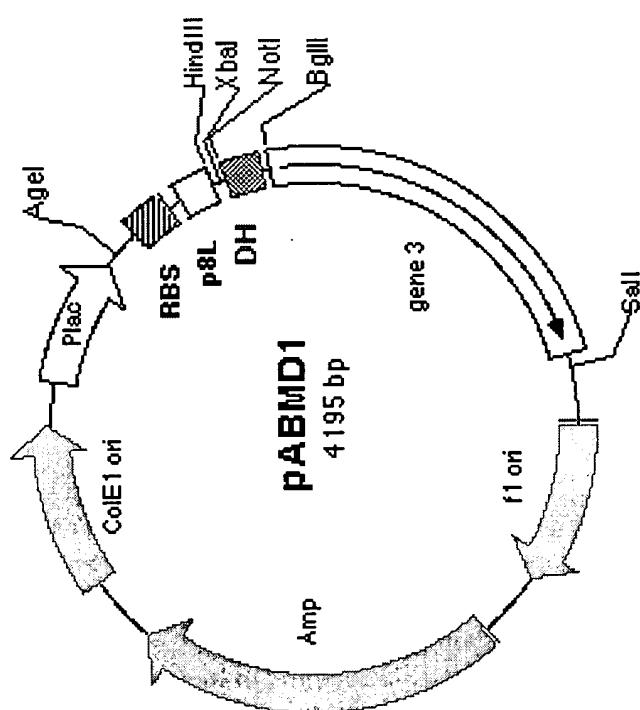
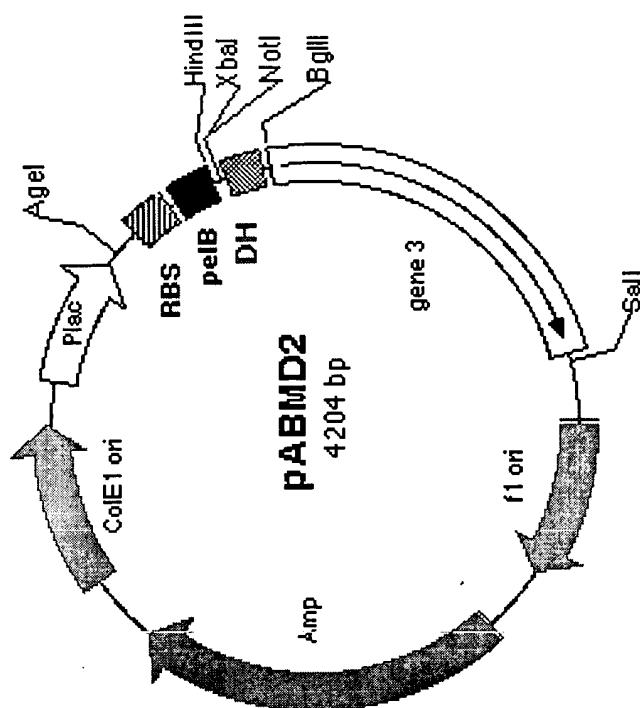


Fig. 22A

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PABMD1 vector: sequence from Agel to Sall

PABMD2 vector: sequence from Agel to Sall

lac promoter/lac O1	Agel	EP	S/D
AATGTTGAGCGGATAACAATT	ACCGGT	TCTT	TTAACCTTAG
pelB Leader			TAAGGAGG
ATGAAATAACCTATTGCCTACGGCAGCCGCTGGATTGTTATTACTCGGGCCCCAGCCGGCCATGGGGCCCTGCAGGGCTCTAGA			AATTTAAAAA
M K Y L L P T A A G L L L A A Q P A M A L Q A S R			
NotI	HA-tag	His-tag	Amber stop
GCGGCCGCT	TATCCATACGACGTACCAAGACTACGCA	GGAGGT	BglII
A A A Y P Y D V P D Y A G G H H H H H * R S			
Gene 3			
GGAGGGGGT	ACTGTTGAAAGTTAGCAAAA	-----	GCTAACACATACTGCGTAATAAGGAGTCTAA
G G G T V E S C L A K			GTCGAC

FOOTER: FIGURE 23

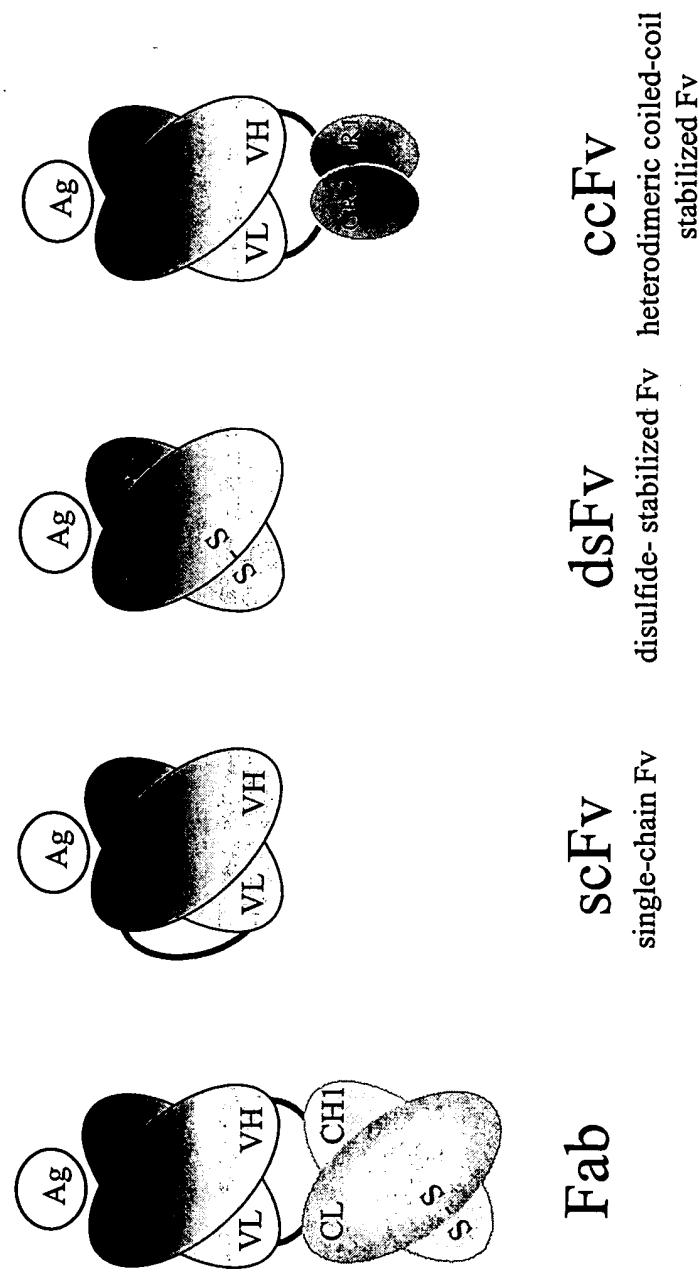
GR1 Sequence Range: 1 to 146

XbaI 10 20 30 40 50
TCTAGAGGTGGAGGAGGTGAGGAAAGTCCGGCTGTTGGAGAACGGAGAA
S R G G G E E K S R L L E K E N
60 70 80 90 100
CCGTGAAGTGGAAAGATCATTGCTGAGAAAGAGGGCTCTGTGAAC
R E L E K I I A E K E E R V S E
110 120 130 140 Ascl
TGCGCCATCAACTCCAGTCTGTAGGAGGTGTTAATAGGGCCGCC
L R H Q L Q S V G G C * *

GR2 Sequence Range: 1 to 140

XhoI 10 20 30 40 50
TCTCGAGGGTGGTGGAAACATCCGGCCTGGAGGGCTACAGTCAGAAA
S R G G G G T S R L E G L Q S E N
60 70 80 90 100
CCATCGCCTGCGAATGAAGATCACAGAGCTGGATAAAAGACTTGGAAAGAGG
H R L R M K I T E L D K D L E E
110 120 130 NotI 140
TCACCATGCAGTGCAGGACGTGGAGGTGCGGCCGCC
V T M Q L Q D V G G C A A A

Fig. 23



Ag antigen

Fig. 24

Expression vector for Adapter-directed bacterial display

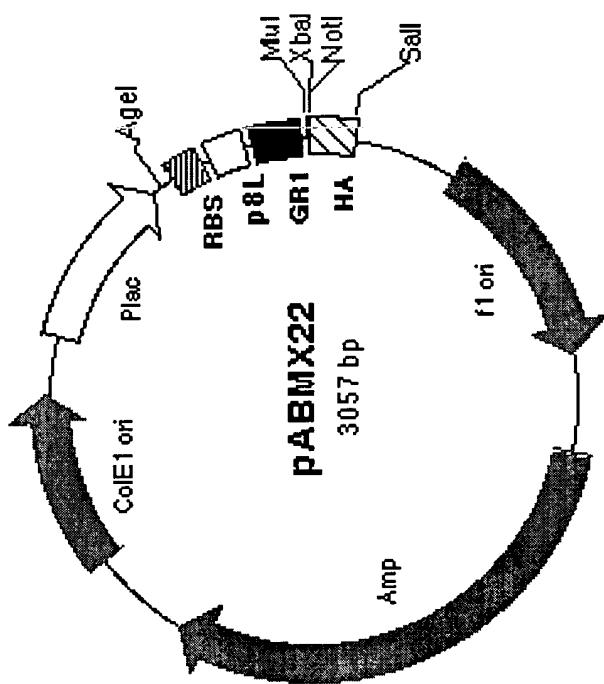


Fig. 25A

Complete vector sequence of pABMX22

GCGAACGCAATTAAATGTGAGTTAGCTCACTTCAAGGACCCCAAGGTTTACACTTTATGCTTCCGGCTCGTATGTTGTGAGGGATAAACAACTTACCGGTTCTTAAGGAGGATT
 AAAAATGAAAGCTTCTTCTGCTCTCAAGGCTCCGTAGCCGGTGTCTACCCCTCGTCCATGCTAAAGTTCGCTGGTCTGCTAGGAAAGAGAACCGTGAACGGAAAAGATC
 ATGGCTGAGAAGAGGGCGCTGTTCTGAACTGCGCCATCAACTGCGAAGTCTGCTGAGTCGCTGTTCTGAACTGGGGCTGCTTCAAGGGGGCTGCTTCAAGGGGACTA
 CCAATTGCCCCATATGTGAGTCGCTGTTCAAACTTCAACTGCGCTGCTGTTCTGAAACCGTCTGCTGTTCAACTGGGGCTGCTTCAAGGGGGCTGCTTCAAGGGG
 ATAGGCGAAAGGGCCCGCACCGATGCCCTCCAAACGGTGGCAAGCTGCTGAACTGGGGCTGCTTCAAGGGGGCTGCTTCAAGGGGGCTGCTTCAAGGGGACTA
 ATTTGCCCCAGGCCCCCTAGGCCGCTCCCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTG
 ACTCTGACCCAAAACCTTATGGGTATGGTCACTGAGGGCTTCACTGGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTG
 ACACACTCAACCCATATCTCGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTG
 TACATTAGTGGCACTTCACTTCCGCTTCACTTCCGCTTCACTTCCGCTTCACTTCCGCTTCACTTCCGCTTCACTTCCGCTTCACTTCCGCTTCACTTCCG
 AGGGAAAGGTATGAGTATGAGTATGAGTATGAGTATGAGTATGAGTATGAGTATGAGTATGAGTATGAGTATGAGTATGAGTATGAGTATGAGTATGAG
 CACGAGTGGGTACATGAACTGGATCTCAACAGGGTAACTTCCGCTTCACTTCCGCTTCACTTCCGCTTCACTTCCGCTTCACTTCCGCTTCACTTCCG
 GCGCCGGGAGGAACTGGTGGCTGCTGAACTTCTGAGTAACTTCTGAGTAACTTCTGAGTAACTTCTGAGTAACTTCTGAGTAACTTCTGAGTAACTTCT
 ACCATGAGTATAACACTGGCCCAACTTACTCTGAGTAACTGGGGATCAACCTGGGGATCAACCTGGGGATCAACCTGGGGATCAACCTGGGGATCAACCTGG
 ATGAAAGGCCATCCAAAGG
 GGGATAAAGTGGAGGACACACTCTGGCTCGCCCTTCCGGTGGTTATGCTGATAAACTGGGGCTGGTGAATGCTGAGTAACTGGGGCTGGTGA
 CTCCCGTATCCGTGTTCTACAGAGGGGAGTCAGGGCAACTATGGGATGAGGAACTATGGGATGAGGAACTATGGGATGAGGAACTATGGGATGAG
 TACTTGTAGTTGATTTAAACCTCATTTTGTGATAATGGTGAATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG
 AGATCAAAAGATCTCTGAGATCTTCTGAGTCTTCTGAGTCTTCTGAGTCTTCTGAGTCTTCTGAGTCTTCTGAGTCTTCTGAGTCTTCTGAGT
 ACTGGCTCAGG
 CTCCCGTACGGCATATGCTG
 GFACTGAGATACCTACAGGCTGAGCTATGAGAAGGGAGAAAGGGAGAAAGGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG
 CCCTGGTATCTTATAGTCTCTGCTGGGCTTCTG
 CCTTTGGCTGGCTTGGCTTGGCTTGGCTTGGCTTGGCTTGGCTTGGCTTGGCTTGGCTTGGCTTGGCTTGGCTTGGCTTGGCTTGGCTTGGCT
 GTAGGGAGG

Helper vector for adapter-directed bacterial display

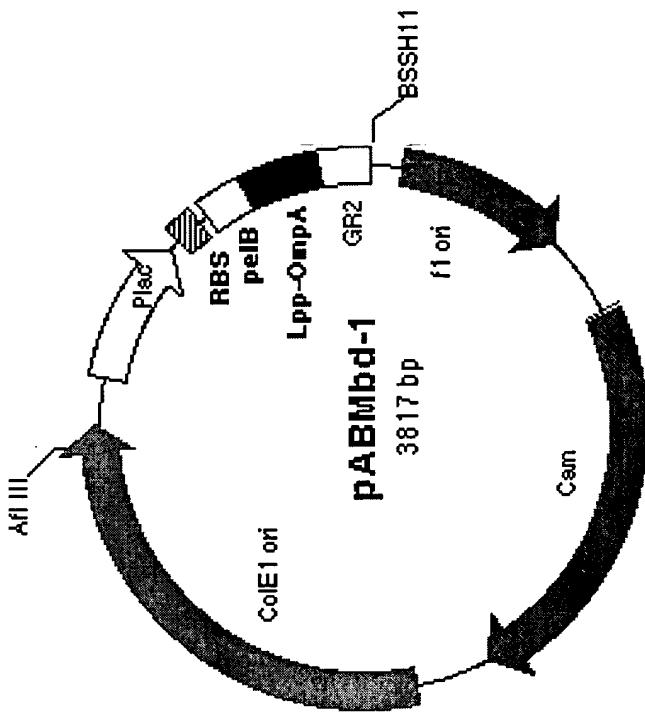


Fig. 26A

Complete vector sequence of pABMXbd-1